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Complementing cooling: the ongoing search for an effective adjunct to therapeutic hypothermia

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Running Title: Adjunct Therapies to Hypothermia

Hypoxic-ischaemic encephalopathy (HIE) remains a leading cause of neonatal death and adverse neurodevelopmental outcome, affecting 2-4 in 1000 live births. In cases of moderate to severe HIE, the current standard of care in near-term or term infants is therapeutic hypothermia initiated within 6 hours of the asphyxic insult. Preclinical and clinical studies have been fundamental in establishing the optimal temperature reduction (3°-5°C) and treatment duration (up to 72h); and importantly have shown that longer or deeper cooling protocols do not improve neuroprotection and in some instances have even demonstrated decreased efficacy. Furthermore, as 8-9 infants need to be treated with optimised therapeutic hypothermia for one infant to survive and/or reduce long term neurodisability

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(Edwards *et al.*, 2010), there remains a clear need to identify adjunct therapies that can further improve outcomes for this vulnerable population.

One such adjunct therapy is erythropoietin (Epo), which has been demonstrated in preclinical studies and a small number of clinical studies to reduce cellular signs of brain injury, macroscopic evidence of injury as assessed by MRI, and result in trends towards improved clinical outcomes in HIE. Moreover, it is an attractive neuroprotective agent as it has an essential role for normal neurodevelopment and has been implicated in injury repair mechanisms through eliciting acute (anti-apoptotic, anti-inflammatory, anti-oxidant and anti-excitotoxic) and regenerative (angiogenesis, neurogenesis, oligodendrogenesis) mechanisms. In addition, the pharmacokinetics of high dose recombinant (rEpo) are now well defined and importantly, it has also been shown to be safe when given in combination with hypothermia in neonates with HIE (Wu *et al.*, 2012).

In this issue of *The Journal of Physiology*, Wassink et al., (2020) investigate whether high-dose rEpo combined with moderate hypothermia in a fetal sheep model of severe global cerebral ischaemia provides added neuroprotection over hypothermia alone. To ensure clinical relevance, their protocol included delayed onset of both hypothermia and treatment, with the infusion of high-dose rEpo initiated 3 hours after the ischaemic insult. Importantly, whilst independently neuroprotective in comparison to the ischaemia-vehicle, Wassink et al., (2020) found there was no enhanced benefit from combining rEpo with therapeutic hypothermia on assessment of both physiological parameters (EEG, heart rate, carotid artery blood flow) and cellular histology. Of added concern, ischaemia-rEpo-hypothermia was also associated with increased cortical caspase-3 positive cells, suggesting increased ongoing cell death even after 7 days of recovery in the combined therapy group.

Although the results of Phase 3 clinical trials of rEpo are still awaited, this study highlights existing concerns about its efficacy as an adjunct clinical therapy for HIE. Along with rEpo, several other agents have also been identified through preclinical studies as promising post-natal adjunct therapies to compliment hypothermia (Robertson *et al.*, 2012; Wassink *et al.*, 2019). However, to date

unfortunately none have been able to demonstrate significant effects as adjunct agents with therapeutic hypothermia in a large clinical trial. Xenon, a monoatomic gas which readily crosses the blood-brain barrier, was found to be of significant benefit in preclinical studies but when taken to a randomised control trial, there was no added benefit when inhaled Xenon was combined with therapeutic hypothermia (Azzopardi *et al.*, 2016). Melatonin similarly is known to cross the blood brain barrier and is an endogenous hormone which is thought have a key role in neural repair through reducing oxidative stress. Although a single small clinical study (Aly *et al.*, 2015) described significant improvements in MRI white matter appearances and short term developmental outcome, a larger scale clinical trial has not been completed. Moreover, the promising results of pre-clinical piglet studies should be considered with caution as effectiveness with delayed initiation of treatment and the potentially adverse effects of the ethanol used as a diluent to deliver melatonin have not been studied in detail (Robertson *et al.*, 2013; Davidson *et al.*, 2015). There are also several clinical trials in progress of autologous stem cells derived from cord blood or the placenta, although their precise mechanism of action for this application is still unclear and thus defining their optimal use remains challenging (Bennet *et al.*, 2012).

So for now, as the search continues, where to next? It is important to consider that the success of therapeutic hypothermia was achieved through a bench to bedside translational pathway which included first establishing the cellular nature and delayed natural history of the underlying brain injury, the mechanisms of potential neuroprotection and then the optimal delivery of the therapy. A vital step in this pathway is therefore to also have preclinical models and protocols which can recapitulate the conditions and standards of clinical care to maintain relevance. Through this, it is likely that the key to identifying the next adjunct therapy will be to understand how the mechanism of action of any potential agent interacts with and hopefully directly complements hypothermia; rather than acting through the same biological route and potentially competing/interfering with hypothermic neuroprotection as appears to be the case with rEpo. Any novel therapy must also be feasible to deliver in the clinical environment, have a favourable risk/benefit ratio, all within the context of significantly improving outcome above and beyond a proven successful therapy.

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Additional Information

Conflicts of Interests

None declared.

Author Contributions

Both authors have equally contributed to conception, drafting and revising the manuscript, having read and approved the final version. They agree to be accountable for all aspects of the work.

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